NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF DIABETES & DIGESTIVE & KIDNEY DISEASES
NATIONAL KIDNEY DISEASE EDUCATION PROGRAM

LABORATORY WORKING GROUP

Conference Call
December 1, 2003

Participants:

Tom Hostetter, MD –NKDEP Director
Elisa Gladstone, MPH – NKDEP Associate Director
Gary Myers, PhD –CDC
Neil Greenberg, PhD – Ortho Clinical Diagnostics
Ethan Hausman, MD –FDA
Tim Larson, MD – Mayo Clinic
Harvey Kaufman, MD – Quest Diagnostics Nichols Institute
Greg Miller, PhD – Virginia Commonwealth University
Michael Welch, PhD – NIST
John Eckfeldt, MD, PhD – University of Minnesota
Jim Fleming, PhD – Lab Corp

Call handouts

Agenda
Dr. Miller's Fresh Frozen Serum PowerPoint presentation
Manuscript outline detailing NKDEP Lab group findings and recommendations
Methodology study submitted by Dr. Greenberg

WECOME AND OVERVIEW

Dr. John Eckfeldt, chair of the lab working group started the conference call.

Representation on the Lab Working Group

Dr. Hostetter provided overview of representatives on the Lab Working Group. Organizations represented are: IFCC, AACC (Tim Larson), CAP (Anthony Killeen), Avomed (Neil Greenberg) CDC (Gary Myers), FDA (Ethan Hausman) and Jim Fleming and Harvey Kaufman from industry. Dr. Eckfeldt would like Dr. Panteghini to participate. Since Dr. Panteghini is in Italy, future calls should be scheduled at 10:00 or 11:00 a.m. While Sharon Burr is currently the representative for CAP, Dr. Eckfeldt would like to have Dr. Anthony Killeen participate as a member.

CAP Linearity proficiency testing material

Dr. Eckfeldt reported that bids were submitted by three vendors in mid-November. Solomon Park's President Dr. Patrick Clapshaw seemed to be the most knowledgeable vendor and appeared to be price competitive. However, it wasn't clear that Solomon Park understood the organization of the on-going basis of this CAP Linearity Study. The final revised bid has not come back because of a misunderstanding of the volume of material which would be needed on an on-going basis. Pilot studies will be run to see how it works as a CAP linearity material. An overrun of this batch of pilot material is what would be used to make NIST/NIH reference material. However, reference material would not be made every year, only enough would be made for the CAP linearity survey. Initially, based on market surveys, CAP anticipates that only about 150 labs will be using this material. Firm pricing and revised specifications are due back to CAP the week of December 1, 2003. Materials should be made in next 3-4 months. The material production protocol will come from the cholesterol reference materials with minor modifications. The two reference material pools are targeted at 0.8 mg/dL creatinine (normal female pool) and this same female pool supplemented with creatinine analytical reagent grade creatinine to a target concentration of 4.0 mg/dL. The high and low pools will be value-assigned with an isotope dilution/mass spectrometry by NIST. NIST would be given 1200 vials for value-assignment which would then they would be sold as a NIST SRM. Hopefully, the reference materials will be prepared and value assigned by summer, 2004.

CAP fall 2003 fresh frozen serum project results

Dr. Miller reported information on the CAP Chemistry Survey which was distributed fresh frozen serum sample in October, 2003, to all of their Chemistry Survey participants. The fresh frozen material had been prepared using the NCCLS – C37 protocol without any supplements whatsoever added. The PowerPoint figure distributed along with the call agenda by Dr. Miller represents the results for creatinine from the participating laboratories. The figure shows the peer group mean bias against the HPLC-established target value (0.90 m/dL), which was the average from multiple measurements made in two different reference laboratories. The bar graph shows the mean bias segregated by CAP-defined peer groups (listed on right side of graph). From left to right, the peer groups have been grouped into four blocks: non-kinetic Jaffe methods, enzymatic methods, kinetic Jaffe methods, and rate blank Jaffe methods. The HPLC-determined reference value was slightly higher than IDMS value on the material, but IDMS analysis was performed in only a single lab. The figure confirms that clinical lab creatinine results are similar to those seen in 1994 CAP fresh frozen serum study, and in general the majority of clinical lab methods are biased high compared to high level reference methods. However, a few methods appear to be well calibrated which is the concentration range critical for calculating a GFR. This graph is a snapshot of current field performance among laboratories across the US. The graph represents data from 5,000 labs, (80% of participant data). Peer groups size range from 20 – 1000. The largest peer group is about 1,000, but most are under 100. This information will be

published in the CAP 2003 Chemistry Survey Participant Summary Report for the C mailing.

Dr. Greenberg revealed that one of the reference labs was his company's (Ortho Clinical Diagnostics). He indicated that the HPLC reference method value reported to CAP was one that was obtained after a modified calibration which they first began using in May, 2003. Previous experience shows that not using protein matrix in the calibrators, results in an even larger bias when their HPLC method's results were compared to IDMS. This modification introduced was to use creatinine in 7 percent BSA rather than water. With BSA-based calibrators, their HPLC method's bias to IDMS was reduced to about 0.05 mg/dL (HPLC giving higher results).

In the future, it will be critical to understand HPLC reference method's bias relative to IDMS, if HPLC is to be used as the basis for calibration of clinical methods. This question is fundamental to the Working Group's mission. At some point, there needs to be an agreement on the reference method used for traceability of clinical laboratory results. Any change in clinical laboratory method calibration will affect the MDRD equation which was been established based upon the Beckman CX3 kinetic Jaffe method. The Beckman CX3 kinetic Jaffe method in the current CAP fresh frozen serum survey and in 1994 were both biased high relative to IDMS by about 0.10 mg/dL. Once the pilot CAP linearity product is approved and manufactured, these specimens could be used in a 'round-robin' between the IDMS and HPLC reference labs, to try to determine the bias and if the bias can be eliminated by more appropriate calibration of both methods.

One point that supports use of the HPLC method is its portability across different laboratories. The ability to have an HPLC reference method in place in a variety of laboratories is much greater than IDMS, although IDMS equipment is becoming more commonly available. Dr. Miller is happy to share the protocol of HPLC, if there is an opportunity to improve upon the HPLC methodology, this would benefit everyone.

Jaffe vs. 'true' creatinine bias across different individuals

Dr. Greenberg discussed the graph that he provided that shows how a kinetic Jaffe method and the Ortho Clinical Diagnostics' Vitros enzymatic creatinine vs. HPLC several years ago. Data set is truncated to focus on low-end activity (<4.0 mg/dL). Both methods show interesting degrees of scatter in terms of bias to HPLC. Unfortunately, Dr. Greenberg is unable retrieve medical records and trace any reasons for the occasionally large biases. Overall, the Jaffe appears higher than HPLC, compared to the enzymatic method, but Dr. Greenberg believes this is mainly related to the two methods' calibration. The enzymatic method has a positive bias, but this bias to HPLC was purposely introduced into the calibration process due to market issues. Dr. Greenberg noted both methods showed occasional "fliers" which cannot be explained. It's difficult to render a judgment about which method is better, at least based on this relatively undefined sample set. Samples containing known interfering substances, such as the ketoacids, were not believed to have been targeted. Perhaps, if a more carefully designed study should be undertaken going after the known interfering substances by the

Laboratory Working Group. Similar studies have been done, but that data is more than 20 years old. Past studies showed that Jaffe methods, which were largely non-kinetic, averaged much higher values than the enzymatic methods.

The purpose of presenting this data was to determine what specimen-specific bias looked like on the comparisons. Two relatively old papers (T. Rosano et al. Clin Chem 1990;36:1951- 5 and Paroni, et al, Clin Chem 1990; 36:830-6) cited by Dr. Eckfeldt show comparisons of HPLC and enzymatic on specific clinical specimens. After looking at the papers, Dr. Eckfeldt is not convinced that specimen-specific biases are any smaller for enzymatic methods, than for the kinetic Jaffe methods as compared to HPLC results. The Sy.x and correlation coefficients are essentially the same when comparing either method to HPLC. All in all, it appears that the kinetic Jaffe's methods appear to do a fairly credible job in terms of improving the Jaffe method's analytical specificity for creatinine. This data suggests there may not be a need to go to more expensive enzymatic methods.

JCTLM – Secondary reference materials and reference methods

Dr. Welch is gathering input from various sub-committees and is planning to put results on the website. Some materials from BCR, NIST, and Korean Standard Organization are on the list. The Korean material is frozen and is one-level, the others are lyophilized serum materials. Three methods used IDMS methods from the following labs: Dr. Lothar Siekmann (Germany), Dr. Linda Thienpont (Belgium), Dr. Welch's at NIST (USA). Dr. Heo (Korea) uses LCMS.

Reference methods have been published and will be sent to Dr. Hostetter. Perhaps the Korean lab using a LCMS reference lab would be willing to share their internal procedure. The Korean lab participated in the international CCQM study.

Manuscript detailing NKDEP's Research and Recommendations

Dr. Myers introduced a proposed outline for the NKDEP manuscript on creatinine calibration. It is modeled after the NCEP/NHLBI Laboratory Standardization Panel's report (the "green book") that provided specific recommendations on improving cholesterol standardization in clinical laboratories. The outline focuses on background of NIDDK and NKDEP, the rationale for generating a new GFR equation, laboratory needs, current performances, analytical specifications needed for estimating GFR, pre-analytical issues affecting creatinine measurements, and analytical issues and recommendations, strengths and weaknesses of the current clinical base systems objective, recommendations for improving and standardizing the measurements, what existing reference methods and materials, external surveillance program, such as the CAP Chemistry Survey, perhaps the proposed CAP Linearity Survey as national resources for creatinine measurements standardizations and improvements. A listing of different organizations involved and potential roles they could play in helping to improve the measurement of creatinine (what NIST, CDC, NIDDK, AACC, CAP, manufacturers could do), could be included in the summary and recommendations.

Unlike the cholesterol report, the NKDEP Lab Working Group anticipates its report will be published in a major peer-review journal. Journal should have a large readership such as *Clinical Chemistry* or *Archives of Pathology and Laboratory Medicine.* It is anticipated that a report from the CAP Chemistry Resource Committee describing the creatinine results of the October, 2003 fresh frozen serum survey will be published in *Archives of Pathology and Laboratory Medicine*. This article could serve as a reference for the NKDEP. Dr. Greenberg noted that the Ontario Proficiency Program published a similar frozen serum creatinine study of Dr. Miller's in 2002, in their association's annual report. Perhaps this could be referenced in the article.

The NKDEP Work Group's manuscript should be kept to 8-10 journal pages of text, excluding references. Possible target journals might include *Clinical Chemistry* and *Archives of Pathology and Laboratory Medicine*. While *Clinical Chemistry* is the most logical, it would be wise to capture as large an international audience as possible. Perhaps the Lab Working Group could do an executive summary and recommendations in the IFCC's journal citing the primary reference in a peer-reviewed journal.

A publication date of as early as June, 2004, for the fresh frozen serum creatinine CAP article, which would be just prior to the AACC national meeting. Hopefully, reprints could be made available for distribution to attendees of the manufacturers' forum at the AACC national meeting.

Dr. Myers agreed to serve as the lead author to collate and edit the various writing groups submissions. Assignments for the overall manuscript were discussed and agreed upon:

Background and rationale - Drs. Hostetter and Eckfeldt

Analytical performance specifications for estimation of GFR - Drs. Greenberg and Hausman

Pre-analytic Issues - Drs. Killeen, Eckfeldt, and Fleming

> Day-to-day variability, specimen, biological variations, special collection conditions, any variables affecting the analytical measurements and results will be covered in this section. Starting point for this section could be reference from *The Kidney*, Brenner and Rechter which gives a decent summary of older literature that discussed the Jaffe interferences. Dr. Hostetter has the 5th edition and will get the 6th edition for others to read.

Analytical issues and recommendations

Clinical laboratory-based analytical systems - Drs. Kaufmann, Hausman, and Greenberg

Recommendations for Standardization - Drs. Myers and Welch

Topics will include reference method of higher order, primary calibration materials, secondary reference materials, currently available reference materials

External surveillance programs - Dr. Miller

National resources for creatinine standardization and improvement - Dr. Miller

Organization to mention might include NIST, CDC, NIDDK, various professional societies, and manufacturers.

Summary - Dr. Myers and Eckfeldt

Timeline: Authors are asked to have drafts of their sections to Gary Myers by mid-February. They should be sent to him by email as Word documents with a cc to Elisa Gladstone. The writing group could then hopefully meet at face-to-face winter meeting to try to pull together a draft of the final article together.

AACC Edutrack

The Edutrack entitled "Detection of Impaired Glomerular Filtration Rate for Assessing Chronic Kidney Disease" has been accepted for AACC meeting and will be presented Thursday afternoon. Dr. Hostetter will give a plenary lecture that same morning. Three speakers will present at the Eductrack: Dr. Miller will provide update on creatinine standardization and calculating GFR, Dr. Josef Coresh (Johns Hopkins) will speak on urinary albumin in the diagnosis of chronic kidney disease, and Dr. Susan Furth, (Johns Hopkins) assessing impaired GFR in children.

AACC manufacturers forum

The manufacturers' forum will be held Monday, July 26 10:00 a.m. to noon. Anticipated audience size and A/V requirements must be confirmed. Based upon cholesterol and hemoglobin A1c standardization forums in the past, Dr. Miller anticipates an audience of 75-100. Presenters at manufacturers' forum should not duplicate the information that will be presented at the Eductrack. Focus of forum should be measurement issues, calibration traceability, and strategies available to manufacturers

In order to promote the forum, the Working Group should collaborate with Industry Division. Rick Miller, Chairman of the Industry Division or AACC should be contacted to confirm any funding and sponsorship of the manufacturers' forum. Although cost only a few hundred dollars, the sponsoring AACC division should still be aware of the obligation. Dr. Fleming and Kaufmann offered to provide the funding for refreshments if they were deemed necessary. Advamed representative, Dr. Greenberg, will take responsibility for forum promotion, including an article if possible in the Division's newsletter and possible the journal *IVD Technology*.

Closing remarks

Dr. Hostetter provided an update on the laboratory suggestion worksheet that was sent out to all ASN members. The NKF and the RPA will also be sending it out. The lab sheet was also provided at the ASN meeting and is posted on the NKDEP website. Anecdotally, there has been a lot of positive response from the orksheet.

Anticipated meeting/conference call schedule for Laboratory Working Group
Conference call – autumn (December 1, 2003)
Meeting – winter (Februrary 25, 2003)
Conference Call – spring (TBD)
Meeting – July 2003 in conjunction with AACC (site/time TBD)

Next Meeting
Wednesday, February 25, 2004
Location near BWI
Anticipate an all day meeting (8:30 am – 3:00 pm)